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Mutations That Destabilize the gp41 Core: Determinants for Stabilizing the SIV/CPmac Envelope Glycoprotein Complex

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Beamline(s): X25

Introduction: The HIV-1 envelope glycoprotein is a primary target for vaccine development because it is the major virus neutralization antigen during natural viral infection. Recent work suggests that an immune response to the native trimeric envelope complex leads to the production of neutralizing antibodies. A major challenge in the development of an effective HIV immunogen is to preserve the native structure in vaccine preparations. An *in-vitro*-derived variant of SIVmac251, denoted CPmac, has been found to acquire an unusually stable gp120-gp41 complex. This unique phenotype is conferred by five amino-acid substitutions in the gp41 ectodomain. To lay the groundwork for future efforts to develop antigenic mimics of the native envelope structure for neutralizing antibody induction, we sought to delineate the role of the CPmac mutations in the folding, thermodynamics, and conformation of the gp41 ectodomain.

Methods and Materials: Diffraction data on NCmac-N40(L6)C38 and CPmac-N40(L6)C38 were collected at 100 K at beamline X25 at the Brookhaven National Laboratory National Synchrotron Light Source using a Brandeis B4 CCD detector. The structures were determined by molecular replacement using the program AmoRe.

Results: To better understand the chemical basis of destabilization of the six-helix bundle by the CPmac mutations, the X-ray crystal structures of the NCmac- and CPmac-N40(L6)C38 proteins were determined. The NCmac-N40(L6)C38 structure was refined to an R-value of 23.7% with an R_{free} value of 28.3% over a resolution range of 50.0 to 2.3 Å. The CPmac-N40(L6)C38 structure was refined to 1.7 Å to yield an R-value of 20.9% with an R_{free} value of 25.2 %. As anticipated, the overall architecture of the CPmac-N40(L6)C38 trimer is the same as that of the wild-type NCmac complex. CPmac-N40(L6)C38 forms a rod-shaped structure of approximately 55 Å in length and 40 Å in diameter. Each polypeptide chain has an α-helical-hairpin conformation, in which two antiparallel helices are connected by a loop region. The N40 helices form an interior, three-stranded α-helical coiled coil. This coiled-coil core includes approximately 34 residues (558-591) (the three most N-terminal and C-terminal residues are disordered). The C38 helices (residues 637-671) pack in an antiparallel manner into hydrophobic grooves on the surface of the trimeric coiled coil (the three most C-terminal residues are not well defined in the electron density maps). The N terminus of N40 and the C terminus of C38 are oriented at the same end of the rod. This packing arrangement would place the fusion peptide, located immediately before N40, and transmembrane segment, located immediately after C38, close together. The structural differences between the NCmac and CPmac six-helix bundles are small: the average r.m.s. difference in Cα positions of the N40 coiled coil between the two molecules is 0.31 Å. The C38 helices can also be superimposed, with an r.m.s. deviation of 0.48 Å. Thus, the five CPmac mutations do not significantly alter the six-helix bundle structure.

Conclusions: Thermal unfolding studies show that the CPmac mutations destabilize the SIVmac251 six-helix bundle by 15 kJ/mol. These results suggest that triggering formation of the fusion-active hairpin structure is thermodynamically coupled to the conformational stability of the native envelope glycoprotein. Thus, introduction of mutations to destabilize the six-helix bundle leads to the stabilization of the trimeric gp120-gp41 complex. This study suggests a potential strategy for the production of stably folded envelope protein immunogens for HIV vaccine development.

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